THE EMERGENCE OF BACTERIAL GENETICS

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> tradict the hypothesis that a specific gene may be entirely responsible for it. But in the case of adaptive enzymes, there is proof that the substrate plays an important and decisive part in the synthesis, although specific genes are implicated in determining the competence to adapt (Monod, 1950).

One of the first studies that Monod did when he moved in 1945 from the Sorbonne to the Pasteur Institute was to distinguish between mutation and enzyme adaptation in *Escherichia coli mutabile*. We have discussed this organism earlier and have noted that it provided the first evidence of a classical mutation mechanism in bacteria (see Section 4.4). Monod and Audureau (1946) isolated a new strain of this organism[9] and confirmed the earlier work that showed that the transformation of lac^- to lac^+ was due to a mutational event. However, Monod went further and showed that the ability to utilize lactose in the resulting lac^+ mutants was due to the formation of an adaptive enzyme. The activity of the enzyme, called at that time *lactase*, was measured using cumbersome oxygen uptake assays in a Warburg apparatus. Glucose-grown cells did not respire when suspended in lactose-containing buffer, whereas lactose-grown cells did. In addition, lac^+ but not lac^- strains exhibited diauxie when cultured in mixed glucose/lactose medium. Monod and Audureau concluded that these results were in accord with Spiegelman's (erroneous) plasmagene hypothesis and Emerson's model of gene action (see Section 10.3).

This work was reviewed extensively by Lwoff (1946) at the important 1946 Cold Spring Harbor symposium, a symposium that Monod also attended. A key statement of Lwoff was:

> In the case of the L^- to L^+ mutation it is reasonable to assume that the change consists of a structural modification of a precursor, which makes it "adaptable" to lactose...This, however, brings up the problem of the mechanism of enzymatic adaptation, which is obviously of greatest importance to our understanding of enzymatic mutations [after mentioning Yudkin, Lwoff then proceeds to present Monod's hypothesis]. Enzymes allowing attack of carbohydrates by bacteria are all derived from a *common precursor* [italics in original]. This precursor (preenzyme) has a slight general affinity for carbohydrates. Transformation of the precursor into an adapted, specific enzyme occurs as a result of the substrate-preenzyme combination (which would account for the specificity of enzymatic adaptation).
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> Certain substrates might have greater affinity for the preenzyme and thus be able to displace other substrates from it, which would explain the *nonspecificity* of the inhibiting effect of "constitutive" substrates (Lwoff, 1946).

This preenzyme hypothesis, based on immunology, would dominate Monod's research for the next five or six years.

In 1948, an important American visitor, the immunologist Melvin Cohn, came to Monod's laboratory. Cohn was to remain in Paris for seven years and was to be a major participant in the "new" thinking that was to develop (Cohn, 1989).

In 1948, Monod reviewed the work on enzymatic adaptation at a symposium organized by Lwoff. This symposium, entitled "Biological units capable of genetic continuity" was one of the first postwar genetics meetings held in Europe. The main motivation for the meeting appears to have been the recent excitement about cytoplasmic inheritance, since this was the main thrust of the talks, which included Sonneborn (*kappa*), Rhoades (plant plas-

[9]Strain ML became the forerunner of a whole group of strains that played an important role in both enzyme induction and in the discovery of bacterial "permeases" (see below).

tids), Bawden (plant viruses), Darlington (plasmagenes), and Brachet (role of plasmagenes in development). However, numerous "microbial" papers were given, including Taylor and Hotchkiss on pneumococcus transformation, Boivin on transformation in *Escherichia coli*, Delbrück on phage genetics, Rountree on lysogeny, Ephrussi on respiratory deficient yeast, and Monod on adaptive enzymes. It seems evident that these microbial systems were represented at the meeting because they were thought to relate to the question of cytoplasmic inheritance. "Conventional" bacterial genetics, such as Lederberg's work, was not included.

At this meeting, Monod presented work that showed that enzyme adaptation for lactase only occurred in *growing* cells. Cells inhibited by chemicals, or more specifically by bacteriophage infection (work done with Elie Wollman; Monod and Wollman, 1947), did not synthesize the adaptive enzyme. Monod presented an alternative model to Spiegelman's plasmagene hypothesis, a model that was an important step in his thinking. He stated that although the gene controlled the enzyme, it was unlikely that it was responsible for all the reactions involved in the synthesis of a molecule as complex as an enzyme protein. Rather, the gene was considered to have a guiding role, elaborating only a small part of the protein, "carrying out a specific priming reaction which brings about the organization of *nonspecific* substances that play a common role in the synthesis of all enzymes in the cell, causing these to be laid down in specific oriented structures. This specific function would only need to occur once, after which the primary unit would no longer be needed. One can readily imagine that this primary unit can be a partial replica (unendowed with genetic continuity) of the gene itself..." (translated from Monod, 1949). It is possible to read too much into such speculations, but the idea of a partial gene replica unendowed with genetic continuity might be considered an antecedent of the messenger RNA hypothesis of 1961 (see later).

10.6 LEDERBERG'S WORK ON THE β-GALACTOSIDASE OF *ESCHERICHIA COLI*

The enzyme β-galactosidase has played an important role in studies on gene expression. In his initial work, Monod used a strain of *Escherichia coli*, strain ML, that could not be analyzed genetically. However, almost from the beginning of his work on bacterial mating in *Escherichia coli* K-12, Lederberg had studied the genetics of the *lac* character. J. Lederberg (1947) initially used *lac* as an unselected marker in his crosses (see Section 5.3). *lac*$^-$ mutants were isolated by plating a large number of cells derived from mutagenized cultures on EMB-lactose indicator agar. In the first genetic map published for *Escherichia coli* (see Section 5.3), the *lac* gene was mapped in a location that is almost exactly where it is figured on current maps (Bachmann, 1987). Following up on this work, J. Lederberg (1948) isolated a large number of *lac*$^-$ mutants and recognized phenotypic differences. Esther Lederberg (1948, 1950, 1952) studied these *lac* mutants further, and showed that there were several alleles. She also found a "position effect" in the interaction of two of the *lac* alleles, a discovery that presaged the discovery of polarity and the *cis-trans* effect of Jacob and Monod some years later. Some of these mutants were certainly in other loci than *lac*, since they had marked pleiotropic effects (J. Lederberg, 1948).

The most important consequence of this genetic work is that it focused attention on the nature of the specific enzyme involved in the hydrolysis of lactose. In a paper that was to have far-reaching consequences, Lederberg developed procedures for assaying the enzyme and studying its biosynthesis (J. Lederberg, 1950). Using his contacts with the Department of Biochemistry at the University of Wisconsin, Lederberg obtained the synthesis of a number of synthetic β-galactosides, including the important compound *o*-nitrophenyl-β-D-galactoside (ONPG) (Seidman and Link, 1950). When this colorless substrate was hydrolyzed, the yellow-colored *o*-nitrophenol was released. Thus, ONPG, being chromogenic, could be used for a quick, specific, and extremely sensitive assay for the enzyme β-galactosidase. Using this new assay, Lederberg studied the effect of substrate concentration on the kinetics of the enzyme in both whole cells and cell-free extracts. Because the Lineweaver/Burk plots in whole cells and autolyzed cells were different, he concluded that "permeability" factors influenced the apparent activity of the enzyme. It is interesting that Monod's laboratory "rediscovered" the role of permeability in the activity of the organism some years later (see later and Rickenberg, Cohen, Buttin, and Monod 1956).

But of most importance in the present context was Lederberg's study of enzyme activity in adapted and unadapted cells. He showed that lactose-grown cells had markedly higher activities than cells grown on other substrates, and that glucose-grown cells had lower activity than cells grown on succinate, a further confirmation of the so-called "glucose effect." Another important discovery presented in this paper was that unadapted cells still retained a small amount of enzyme. Monod had used a manometer to assay for the enzyme, but according to Lederberg "Manometric experiments would usually miss activity of the order of a small per cent of adapted cells..." This presence of a basal level of enzyme in unadapted cells should have, but apparently did not, rule out certain of Monod's models for the mechanism of enzymatic adaptation.

In 1950, the Genetics Society of America held a jubilee meeting entitled Genetics in the 20th Century, celebrating the 50th anniversary of the rediscovery of Mendel's laws (Dunn, 1951). At this meeting, Lederberg presented an important paper on bacterial genetics that had an extensive section on *lac* (J. Lederberg, 1951). This paper presented a systematic study of Lederberg's *lac*$^-$ mutants, using the newly available ONPG chromogenic substrate and some synthetic galactosides as possible inducers. Although many of the *lac*$^-$ mutants were clearly pleiotropic, several were directly involved with β-galactosidase. One of Lederberg's mutants, designated *lac*$_1^-$, was found to synthesize minimal amounts of β-galactosidase when grown on lactose but substantial amounts when grown on an alkyl galactoside. In discussing this result, Lederberg made the following prescient statement:

> This results in the paradox that cells grown on a heterologous substrate are better adapted to lactose than those grown on lactose itself. Since "adaptation" is presumably a physicochemical rather than an entelechist process, such deviations are not surprising but suggest the need for revising "adaptive enzyme formation" in favor of a more general term connoting "enzyme formation under environmental influence" (J. Lederberg, 1951).

Subsequently, Monod expressed this same idea when he adopted the term "enzyme induction" (see later) but did not mention Lederberg's prior work (Monod, Cohen-Bazire, and Cohn, 1951).

Another important announcement in Lederberg's 1951 paper was the isolation of the first *constitutive* mutant (which he designated Cst^+). Because such mutants provided one of the strongest points against the somewhat teleological models of enzyme adaptation that had been advanced earlier, it is significant that Lederberg's constitutive mutant was announced at least a year before those of the French group. The manner in which Lederberg isolated the mutant was also interesting. *Escherichia coli* did not attack the sugar neolactose; Lederberg attempted to isolate a mutant with altered specificity to β-galactosides by growing the cells on this neolactose. Such a mutant was readily isolated; then it was found that β-galactosidase could split neolactose but that the latter was unable to induce the formation of the enzyme. It was then found that the adaptive system had been changed to a constitutive one. Excellent β-galactosidase production was obtained even when the mutant was grown on glucose. Lederberg concluded that substrate-dependent β-galactosidase formation was subject to rather complex genetic control (J. Lederberg, 1951).

In this paper Lederberg thus presented the key ideas that later became the Monod canon: (1) certain β-galactosides are substrates of β-galactosidase but not inducers; (2) certain β-galactosides are inducers and are not substrates; (3) mutants can be isolated that do not form β-galactosidase because of altered regulatory properties, rather than because of alterations in the structural gene of the enzyme (constitutive mutants); (4) an adaptive enzyme can be synthesized in the absence of its substrate, so that the β-galactoside must be viewed as a regulatory substance as well as a substrate.

These ideas were not very strongly presented, and the data to support them were almost "throwaways." Furthermore, much biochemical work would be needed to rule out any alternative explanations,[10] but these results provided the essential leads for the subsequent work of Monod and his collaborators. Why were they ignored by Monod? Although some chauvinism may have been involved, my contention is that the evidence was genetic rather than physiologic, which was what Monod was seeking. In addition, "Jacques did not like to get rid of his theories. He had a strong tendency to stick to his model, sometimes slightly beyond the point of reason" (Jacob, 1979). Only gradually would the old ways of thinking die out and be replaced by the new.

10.7 FROM ENZYMATIC ADAPTATION TO INDUCED ENZYME SYNTHESIS

In Monod's laboratory, the period from about 1950 until 1955 represented a critical transition in thinking on the problem of enzyme adaptation. An overview of this period can perhaps best be obtained from Melvin Cohn's Eli Lilly Award address (Cohn, 1957), whose main points are summarized here.

Constitutive Mutants

The isolation of constitutive mutants placed the problem of the role of the substrate in a new light. With Lederberg's work as a background, Cohen-Bazire and Jolit (1953) set out to isolate constitutive mutants in Monod's laboratory. Instead of neolactose, they used lactose but adopted a cycling

[10]It was necessary, above all, to show that constitutive mutants produced the *same* enzyme protein as the wild type.

procedure in which the culture was grown repeatedly in a glucose medium and then transferred into lactose medium. Because the wild type lacked β-galactosidase when grown in glucose, there was a latent period in the lactose medium before growth was resumed. However, constitutive mutants present in the culture would begin growth immediately, so that at the end of the latent period the culture would be enriched in constitutive mutants. This "adapted" culture was then transferred back to glucose medium, where both the wild types and the constitutives grew normally. Constitutive mutants eventually dominated and could be purified by streaking on plates.

Although J. Lederberg (1951) had interpreted constitutive mutants as due to changes in regulatory properties, the Monod laboratory subscribed to a different theory, hypothesizing that these mutants produced an *internal inducer*. The hypothesis of an internal inducer was in line with the strong feeling of Monod (1947) that a unitary hypothesis was necessary to explain adaptive and constitutive enzyme synthesis. Why was an internal inducer needed for *every* adaptive enzyme in the cell? Monod had obviously not yet thought of the more economical (and correct) hypothesis of a negative regulatory system that was abolished in constitutive mutants. A genetic consequence of the internal inducer hypothesis was that if complementation tests were run, constitutivity should be dominant over inducibility. At the time, *Escherichia coli* genetics was not far enough advanced to permit complementation tests to be run. Such tests later would readily eliminate the internal inducer hypothesis (see Section 10.10).

Artificial Galactosides

The synthesis of artificial β-galactosides made it possible to separate their role as enzyme substrates from their role as inducers. As noted earlier, J. Lederberg (1950) had developed synthetic β-galactosides as both substrates and inducers. However, his interest was primarily genetic, and he did not pursue this line of work. Melvin Cohn, in Monod's laboratory, would carry the study of synthetic galactosides to its ultimate conclusion. He synthesized a wide array of β-galactosides, among which were several that were excellent inducers but were not substrates of the enzyme. According to Monod's theory (1947), the substrate was to have a directive influence on the aggregation of protein subunits by combining with the active site of the enzyme, leading to the formation of the final product. However, the existence of nonsubstrate inducers ruled out any direct connection between the β-galactoside as substrate and as inducer. This discovery had a profound philosophical impact on Monod, for it ruled out any teleological explanation of enzyme adaptation. This led to the use of a new term, *induction*, to replace the philosophically "loaded" term *adaptation*. The fact that a β-galactoside could be an inducer but not a substrate led to the use of the term *gratuitous induction*, a term meaning that the kinetics of enzyme formation were being studied under conditions such that neither the enzyme nor its inducer influenced general cellular metabolism. Gratuitous induction made it possible to study enzyme synthesis separately from enzyme function, thus making the study of kinetics more meaningful (Monod, Cohen-Bazire, and Cohn, 1951; Monod and Cohn, 1952; Cohn, Monod, Pollock, Spiegelman, and Stanier, 1953).

Precursor Pz

The most widely held model for adaptive enzyme synthesis postulated a conversion of a precursor protein into the final enzyme under the influence of the inducer. This model was first elaborated in detail by Spiegelman (1945, 1946), but had already been conceived independently by Monod, and was presented by Lwoff (1946) at the 1946 Cold Spring Harbor symposium. Subsequently, the precursor model had received some support from the discovery by Cohn and Torriani (1952) of a protein called Pz, present in uninduced cells, that was immunologically related to β-galactosidase (abbreviated Gz). A study was therefore initiated of the possible role of Pz as a precursor of Gz (Cohn and Torriani, 1952, 1953). Since Pz was related immunologically to Gz, it was reasonable to hypothesize that Pz was the postulated precursor. The Pz protein was found in all strains of enteric bacteria that fermented lactose, but was absent from non-lactose-fermenters. Uninduced cells that contained negligible amounts of Gz (just the basal level) contained Pz. Finally, when a gratuitous inducer was added to a culture, the amount of Pz decreased while Gz increased (although the decrease in Pz appeared to be quantitatively less than the increase in Gz).

Pz, however, was one of those sidelines down which research is often drawn. Radioisotope studies would subsequently show that Gz was not formed from Pz, but *de novo* (see below). Pz turned out to have nothing to do with β-galactosidase, and the fact that it cross-reacts immunologically with β-galactosidase is incidental (Pappenheimer, 1979).

The parallel between the Pz model and Spiegelman's model is illustrated in Figure 10.3b.

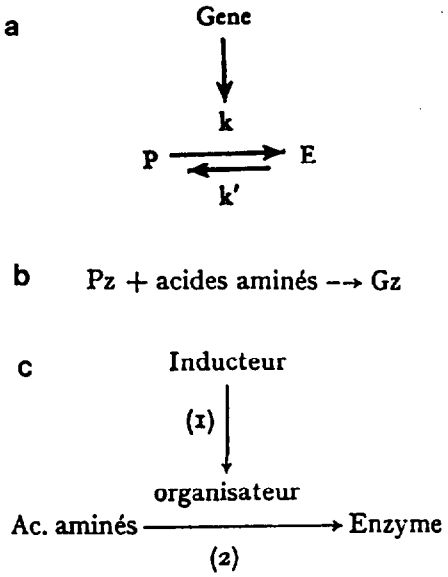

Figure 10.3 Comparison of precursor models. (*a*) Spiegelman's model for adaptive enzyme formation. (Reprinted, with permission, from Spiegelman, 1945.) (*b*) The Pz model of Monod. (Reprinted, with permission, from Monod, Pappenheimer, and Cohen-Bazire, 1952.) (*c*) Pollock's organizer concept, as visualized by Monod, Pappenheimer, and Cohen-Bazire. (Reprinted, with permission, from Monod, Pappenheimer, and Cohen-Bazire, 1952.)